• Basic Research •

Semaphorin 7a participants in pterygium by regulating vascular endothelial growth factor

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Abstract

• AIM: To investigate the relationship between semaphorin 7a expression and cell proliferation and migration in pterygium fibroblasts.

• METHODS: Twenty-six patients with surgically diagnosed pterygium were enrolled, including 15 cases of primary pterygium and 11 cases of recurrent pterygium. In addition, 12 cases of normal conjunctival tissue were collected. The expression of semaphorin 7a in normal conjunctival tissue, primary pterygium and recurrent pterygium was detected by real-time polymerase chain reaction. Recurrent pterygium fibroblasts were isolated and cultured, and the expression of semaphorin 7a was silenced by small interfering RNA (siRNA) interference technique. Furthermore, the effects of si-semaphorin 7a interference on the mRNA and protein levels of β1-integrin, vascular endothelial growth factor A (VEGFA) and vascular endothelial growth factor receptor (VEGFR), and on fibroblast proliferation were analyzed. Transwell assay was used to detect the effect of semaphorin 7a interference on fibroblast migration.

• RESULTS: Semaphorin 7a was highly expressed in the primary pterygium and recurrent pterygium samples than that of the normal conjunctival tissue. Compared with the primary pterygium, the expression of semaphoring 7a in the recurrent pterygium samples was significantly increased (P<0.05). The mRNA and protein expression levels of β1-integrin, VEGFA and VEGFR were decreased after si-semaphorin 7a transfection, and as well as the cell proliferation and migration.

• CONCLUSION: Semaphorin 7a might play important roles in the pathogenesis of pterygium by affecting the expression of β1-integrin, VEGFA and VEGFR.

• KEYWORDS: semaphorin 7a; pterygium; β1-integrin; vascular endothelial growth factor; fibroblast

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INTRODUCTION

Pterygium with a "pterygium belt" is characterized by a conjunctival disease, in which the vascular tissue of the conjunctival fibrosis is invaded by the cornea[1]. It is a common and frequently-occurring disease in ophthalmology. The incidence of pterygium is reported to be between 0.3% and 37.5% in the world[2]. The cause of pterygium is generally considered to be a chronic inflammatory disease caused by external stimuli. It has been reported that the prevalence in people over 50 years old in rural areas of southern China is over 30%[3]. The pterygium affects not only the facial appearance, but also the pterygium tissue may grow into the cornea to lead to corneal astigmatism and vision loss. At present, surgical resection is the main method for the treatment of pterygium in the clinic. However, the recurrence rate is relatively high, up to 20%-40%[4]. Once the pterygium relapses, it grows rapidly, and the treatment is difficult. Moreover, recurrent pterygium can also cause serious complications

such as limited eye movement. Therefore, elucidating the molecular mechanism of recurrent pterygium, and identifying the effective target genes is important for the treatment of pterygium.

Previous studies suggested that neovascularization occurs in pterygium[5-6]. The semaphorins protein family containing a large number of transmembrane proteins and secretory proteins are widely expressed in immune, cardiovascular, and respiratory systems, and play important roles in vertebrate nerves and immune systems[7]. Semaphorins family proteins can be divided into 8 subfamilies based on sequence similarity and structural features[8-9]. Semaphorin 7a, also known as CD108, is the only member of the seventh subfamily of the semaphorins family[10]. Studies have shown that semaphorin 7a induces potent stimuli of monocytes by proinflammatory cytokines[11], and it regulates T cell immune responses *via* mediating T cell proliferation[12]. In addition, semaphorin 7a plays an important role in the migration of osteoblasts and osteoclasts during bone remodeling, and in tumor angiogenesis[13-14]. However, there has been no related study of semaphorin 7a in pterygium.

Previous studies have showed both hyperplasia and degeneration in the epithelial layer and the superficial layer of pterygium epithelium, accompanied by neovascularization and inflammatory cell infiltration[15]. Vascular endothelial growth factor (VEGF) and β1-integrin are important factors involved in angiogenesis[16]. Recently, Boudria *et al*[16] proposed that β1-integrin is required for the invasive functions of vascular endothelial growth factor A (VEGFA), and an invasive VEGFR/β1-integrin loop is required for proliferation and invasiveness of cancers by VEGFA. In this study, the mRNA and protein expression levels of semaphorin 7a in primary pterygium and recurrent pterygium were examined. Primary culture of fibroblasts from recurrent pterygium was performed; the expression of semaphorin 7a was inhibited by small interfering RNA (siRNA); and the expression levels of VEGFA, and vascular endothelial growth factor receptor (VEGFR), and β1-integrin effected by si-semaphorin 7a was investigated. In addition, the proliferation, infiltration and migration of fibroblasts were further studied. The results may indicate the role of semaphorin 7a in pterygium recurrence.

SUBJECTS AND METHODS

Ethical Approval The study protocol was approved by the Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong University and was conducted in accordance with the Declaration of Helsinki. Patients were voluntarily signed informed consent document prior to entry into the study.

Clinical Participants Twenty-six patients diagnosed with pterygium and removed surgically in our hospital from March 2017 to February 2018 were enrolled. There were 15 cases of primary pterygium, including 7 females and 8 males with an average age of 45y (37-59y), and 11 cases of recurrent pterygium including 6 males and 5 females with an average age of 43y (39-56y). The pterygium head extending at least 2 mm into the cornea. The control group was conjunctival tissue of conjunctival or post-traumatic eyeball removed from 12 accidental patients, including 7 males and 5 females, with an average age of 49y (41-65y). All patients had no other corneal or conjunctival disease, and all cases had not received medical treatment before surgery.

mRNA Level of Semaphorin 7a by Real-time Quantitative Polymerase Chain Reaction Total RNA from clinical samples was extracted by TRIzol reagent (Invitrogen, USA), and determined for RNA purity and concentration by OD260/OD280 ratio on spectrophotometer. RNA was reverse transcribed into cDNA using the PrimeScript RT Master MIX (Perfect Real Time) kit (TaKaRa) according to the manufacturer's instructions. Fluorescence real-time quantitative polymerase chain reaction (qPCR) was performed according to the instructions of the SYBR Premix Ex Taq TM II (Perfect Real Time; Sigma, USA). The reaction procedure was as follows: pre-reaction at 50℃ for 2min, 95℃ for 5min, denaturation at 95℃ for 15s, annealing at 60℃ for 60s, a total of 40 cycles; melting curve program was: 95℃, 15s, 60℃, 60s and 95℃, 15s. The real-time PCR primers are shown in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the internal reference, and the relative expression of each gene was calculated by $2^{-\triangle\triangle CT}$.

Protein Level of Semaphorin 7a by Western Blot The pterygium samples were lysed, and detected for semaphorin 7a level by primary anti-semaphorin 7a (1:1000, Cat. No.MA1-19203, ThermoFisher, USA) and secondary antibody IgG-HRP (1:1000, sc-2392, BD, USA).

Isolation and Culture of Recurrent Pterygium Fibroblasts The pterygium tissue was removed from the excised tissue, digested by collagenase at 37℃ incubator for 1h. The cells were filtered, collected, resuspended in medium, and subcultured. The marker of fibroblasts, vimentin, was verified by immunofluorescence staining using antibody specific for vimentin (1:250, ab92547, Abcam, USA). Fibroblasts at passages 2-5 were selected for subsequent experiments.

RNAi of Semaphorin 7a The semaphorin 7a gene sequence was searched from the NCBI website, and the target sequence was designed using siRNA design software. A total of three target sequences were designed: siRNA1, sense: 5'-GGACAAUCCTGACAAGAAU-3'; anti-sense: 5'-AUUCUUGUCAGGAUUGUCC-3'; siRNA2, sense: 5'-GGGCAUGGGUUCUUGGAGA-3'; anti-sense: 5'-UCUCCAAGAACCCAUGCCC-3'; siRNA3,

Table 1 Primers of semaphorin 7a, β1-integrin, VEGFA, VEGFR and GAPDH

Gene	Upstream primer	Downstream primer
Semaphorin 7a	5'-TCATCAAAGCCACCATCG-3'	5'-AGCTCACATACAGCTTCCTCC-3'
β1-integrin	5'-CAAAGGAACAGCAGAGAAGC-3'	5'-GTGGAAAACACCAGCAGC-3'
VEGFA	5'-AGGGCAGAATCATCACGAAGT-3'	5'-AGGGTCTCGATTGGATGGCA-3'
VEGFR	5'-GGCCCAATAATCAGAGTGGCA-3'	5'-CCAGTGTCATTTCCGATCACTTT-3'
GAPDH	5'-AAATCCCATCACCATCTTCCAG-3'	5'-GAGTCCTTCCACGATACCAAAGTTG-3'

VEGFA: Vascular endothelial growth factor A; VEGFR: Vascular endothelial growth factor receptor; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

sense: 5'-CUAAAUACCACUACCAGAA-3'; anti-sense: 5'-UUCUGGUAGUGGUAUUUAG-3'; control sequence, sense: 5'-GUUCUCCGAACGUGUCACG-3'; anti-sense: 5'-CGUGACACGUUCGGAGAAC-3'. The oligonucleotide sequences were synthesized and ligated to the vector. The ligated DNA was transformed into E. coli strain DH5α. Positive clones were picked and plasmids were extracted.

The pterygium fibroblasts (5×10^5 cells/well) were seeded in 6-well plates until the cell fusion degree was 70%-80%. si-semaphorin 7a and negative control si-negative were transfected into fibroblasts according to the instructions of Lipofectamine 2000 Transfection Reagent (Invitrogen, USA), and the transfection efficiency was detected by qPCR.

Effect of Semaphorin 7a Interference on mRNA Expression of β1-integrin, VEGFA and VEGFR by Real-time PCR After 24h of siRNA transfection, fibroblasts were collected. The expression of β1-integrin, VEGFA and VEGFR in each group was detected by real-time PCR. The method was mentioned above. The primer sequences are shown in Table 1.

Effect of Semaphorin 7a Interference on Protein Expression of β1-integrin, VEGFA and VEGFR by Western Blot Analysis After 24h of siRNA transfection, fibroblasts were collected, and the protein levels of β1-integrin, VEGFA and VEGFR in each group was detected by Western blot. The antibodies were anti-β1-integrin (1:2000, ab179471, Abcam, USA), anti-VEGFA (1:200, ab1316, Abcam), anti-VEGFR (1:500, Ab11939, Abcam).

MTT Assay for Cell Proliferation The cells were collected at 24, 48 and 72h after siRNA transfection, and 20 μL of MTT (5 mg/mL) was added to each well (96-well culture plate), and incubation was continued at 37℃, 5% CO_2 for 4h. The optical density (OD) value at 490 nm was measured.

Cell Migration Test The effect of semaphorin 7a interference on the migration ability of fibroblasts was examined using Transwell assay as described elsewhere. The number of cell migration was quantified by randomly counting five independent fields using microscope.

Statistical Analysis Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Each experiment was repeated three times. The *t*-test was used for comparing the two groups, and the one-way analysis of variance (ANOVA) was used for comparing the multiple groups. A $P<0.05$ was considered to be a significant difference.

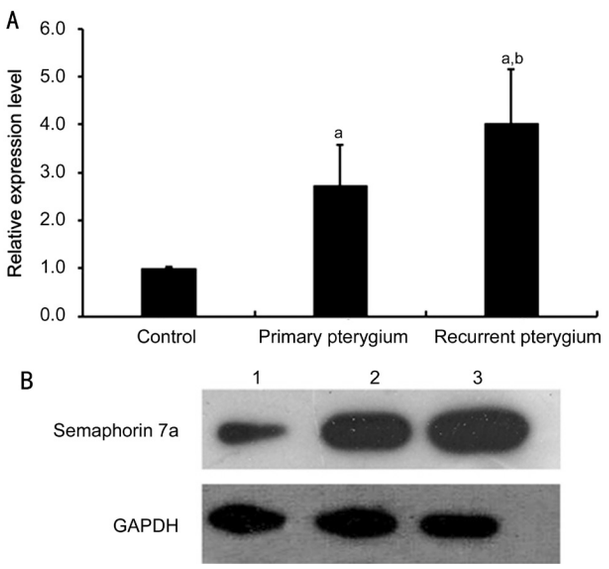

Figure 1 Real-time PCR (A) and Western blot (B) analysis of semaphorin 7a level in normal conjunctival tissue, primary pterygium and recurrent pterygium [a]$P<0.01$ compared with the control; [b]$P<0.01$ compared with primary pterygium. Lane 1: Normal conjunctival tissue; Lane 2: Primary pterygium; Lane 3: Recurrent pterygium.

RESULTS

Expression Level of Semaphorin 7a Increased in Pterygium Samples Real-time PCR results showed that the expression of semaphorin 7a in the primary pterygium and recurrent pterygium samples was significantly higher than that of the normal conjunctival tissue (all $P<0.05$). Compared with the primary pterygium sample, the expression of semaphorin 7a was significantly increased in the recurrent pterygium samples ($P<0.05$, Figure 1A). Western blot results showed that the protein band of primary pterygium was significantly thicker than that of normal conjunctival tissue, and the protein band of recurrent pterygium was significantly thicker than that of primary pterygium (Figure 1B).

mRNA Level of Semaphorin 7a Decreased After si-semaphorin 7a Transfection Fibroblasts of recurrent pterygium were isolated. Vimentin is an essential marker of

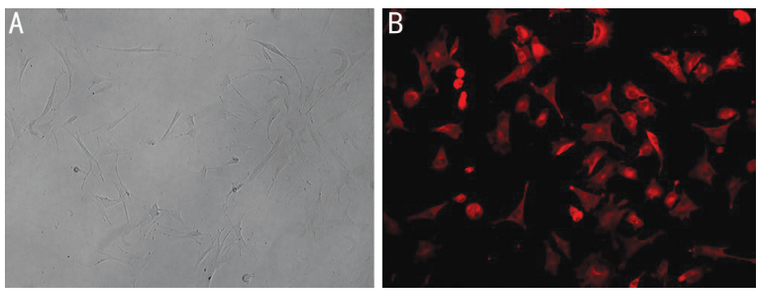

Figure 2 Isolation and characterization of fibroblasts A: Morphology of fibroblasts; B: Immunofluorescence staining of vimentin.

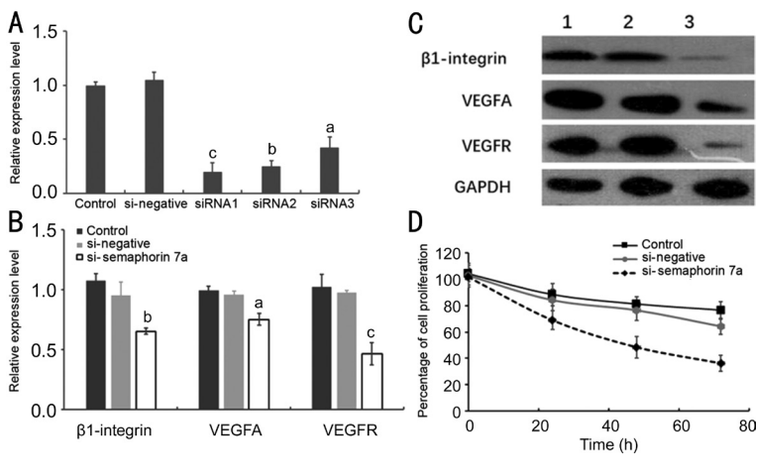

Figure 3 Effect of semaphorin 7a interference on gene expression and cell function of fibroblasts A: si-semaphorin 7a silencing efficiency; B: The effects of semaphorin 7a interference on β1-integrin, VEGFA and VEGFR mRNA expression. [a]P<0.05, [b]P<0.01, [c]P<0.001, respectively, compared with the control group. C: The effect of semaphorin 7a interference on the protein levels of β1-integrin, VEGFA, and VEGFR. Lane 1: Control group; Lane 2: si-negative group; Lane 3: si-semaphorin 7a group. D: The effect of semaphorin 7a interference on fibroblast proliferation.

fibroblasts. To validate the successful of isolation, vimentin was examined by immunofluorescence microscopy (Figure 2). Real-time PCR results showed that the mRNA level of semaphorin 7a in fibroblasts was significantly decreased after si-semaphorin 7a transfection with an siRNA entrapment efficiency of 70%-80% (Figure 3A). Then siRNA1 was selected for further experiments.

Expression Levels of β1-integrin, VEGFA and VEGFR Decreased After si-semaphorin 7a Transfection Treatment of fibroblasts with si-semaphorin 7a for 24h significantly inhibited the mRNA expression of β1-integrin, VEGFA and VEGFR (P<0.05, Figure 3B).

Western blot results showed that the bands of β1-integrin, VEGFA and VEGFR were significantly thinner in si-semaphorin 7a treated group (Figure 3C). The results suggested that semaphorin 7a interference can significantly affect the expression of β1-integrin, VEGFA and VEGFR.

Cell Proliferation Inhibited After si-semaphorin 7a Transfection Compared with the control group, the cell proliferation ability of si-semaphorin 7a treated group was significantly weaker in a time-dependent manner (P<0.05, Figure 3D).

Cell Migration Ability Impaired After si-semaphorin 7a Transfection Transwell results showed that the migration ability of fibroblasts in si-semaphorin 7a transfected cells was significantly lower than that in si-negative treated and control cells (P<0.05, Figure 4).

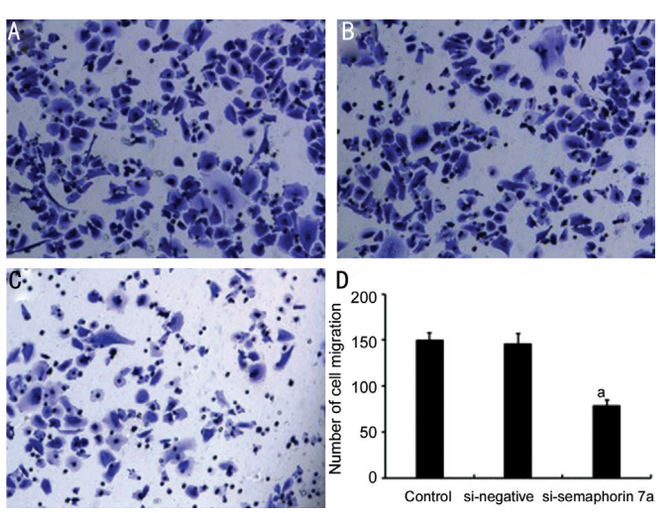

Figure 4 Effect of semaphorin 7a interference on fibroblast migration ability A: Control group; B: si-negative group; C: si-semaphorin 7a group; D: The number of migratory cells in each group. [a]P<0.01 compared with the control group.

DISCUSSION

Pterygium is a chronic proliferative eye disease mainly with abnormally proliferating epithelial cells, fibroblasts and

neovascularization in pterygium tissue[17]. Semaphorin 7a has been reported to be associated with a variety of cancers, such as breast cancer[18], and oral squamous cell carcinoma[19]. Thus, this study aims to identify the relationship between semaphorin 7a and pterygium.

Real-time PCR and Western blot showed that the expression of semaphorin 7a in primary pterygium and recurrent pterygium were higher than that in normal conjunctival tissue, and that in recurrent pterygium samples was highest. Semaphorin 7a showed pro-angiogenic properties is associated with angiogenesis in vascularized corneas[13]. It is also involved in corneal nerve regeneration and inflammation in the cornea[20]. This suggested that semaphorin 7a overexpression may be associated with the recurrence of pterygium, and cell proliferation and migration in pterygium tissues.

In addition, and the expression levels of VEGFA and VEGFR affected by si-semaphorin 7a were also decreased. VEGF as one of the most potent angiogenic factors was suggested to be involved in the pathogenesis of pterygium by triggering angiogenesis in neovascularization[21]. Previous studies indicated that VEGF was highly expressed in the development of pterygium[21-22]. Overexpressed VEGFR in primary pterygia and recurrent pterygia was recognized[23]. In addition, Feng *et al*[24] indicated that β1-integrin related to adhesion and migration of conjunctiva cells participants in the occurrence and recurrence of pterygium. β1-integrin recruitment and VEGFR clustering was found associated with DNA synthesis and cell migration[25]. The β1-integrin/EGFR/VEGFA/VEGFR-1 signaling axis is needed for cancer invasion and metastasis[26]. Semaphorin 7a containing an RGD motif binds to β1-integrin to decreases integrin-mediated cell attachment[27]. Although the indirect VEGF-semaphorin interactions, the expression levels of VEGF- and semaphorin-related genes were highly correlated in breast cancer[28]. Thus after semaphorin 7a interference, the highly expressed VEGFA, VEGFR and β1-integrin could increase the blood supply of pterygium, and thus participated in neovascularization of pterygium, which contribute to the progression and recurrence of pterygium[21,29-30]. Additionally, the proliferation and migration ability of fibroblasts was also decreased significantly after inhibition of semaphorin 7a expression. These results suggested that semaphorin 7a may be a potential target for the treatment of primary or recurrent pterygium.

In conclusion, the expression of semaphorin 7a might be closely associated with the malignant nature of pterygium growth and recurrence. Inhibiting semaphorin 7a in fibroblasts can significantly inhibit the expression of VEGFA and VEGFR, and also reduce the proliferation and migration of pterygium cells significantly.

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REFERENCES

1 Detorakis ET, Spandidos DA. Pathogenetic mechanisms and treatment options for ophthalmic pterygium: trends and perspectives (Review). *Int J Mol Med* 2009;23(4):439-447.

2 Singh H, Laad S, Pattebahadur RS, Saluja P, Ramnani P. A comparative study of postoperative outcome after pterygium excision using autologous blood and sutures. *J Evid Based Med* 2017;4(95):6022-6027.

3 Wu KL, He MG, Xu JJ, Li SZ. Pterygium in aged population in Doumen county, China. *Yan Ke Xue Bao* 2002;18(3):181-184.

4 Fukushima S, Inoue T, Inoue T, Ozeki S. Postoperative irradiation of pterygium with 90Sr eye applicator. *Int J Radiat Oncol Biol Phys* 1999;43(3):597-600.

5 Liu DW, Peng C, Jiang ZX, Tao LM. Relationship between expression of cyclooxygenase 2 and neovascularization in human pterygia. *Oncotarget* 2017;8(62):105630-105636.

6 Mak RK, Chan TC, Marcet MM, Choy BN, Shum JW, Shih KC, Wong IY, Ng AL. Use of anti-vascular endothelial growth factor in the management of pterygium. *Acta Ophthalmol* 2017;95(1):20-27.

7 Pasterkamp RJ, Giger RJ. Semaphorin function in neural plasticity and disease. *Curr Opin Neurobiol* 2009;19(3):263-274.

8 Worzfeld T, Offermanns S. Semaphorins and plexins as therapeutic targets. *Nat Rev Drug Discov* 2014;13(8):603-621.

9 Nogi T, Yasui N, Mihara E, Matsunaga Y, Noda M, Yamashita N, Toyofuku T, Uchiyama S, Goshima Y, Kumanogoh A, Takagi J. Structural basis for semaphorin signalling through the plexin receptor. *Nature* 2010;467(7319):1123-1127.

10 Czopik AK, Bynoe MS, Palm N, Raine CS, Medzhitov R. Semaphorin 7A is a negative regulator of T cell responses. *Immunity* 2006;24(5):591-600.

11 Holmes S, Downs AM, Fosberry A, Hayes PD, Michalovich D, Murdoch P, Moores K, Fox J, Deen K, Pettman G, Wattam T, Lewis C. Sema7A is a potent monocyte stimulator. *Scand J Immunol* 2002;56(3):270-275.

12 Kang SJ, Okuno T, Takegahara N, Takamatsu H, Nojima S, Kimura T, Yoshida Y, Ito D, Ohmae S, You DJ, Toyofuku T, Jang MH, Kumanogoh A. Intestinal epithelial cell-derived semaphorin 7A negatively regulates development of colitis via αvβ1 integrin. *J Immunol* 2012;188(3):1108-1116.

13 Ghanem RC, Han KY, Rojas J, Ozturk O, Kim DJ, Jain S, Chang JH, Azar DT. Semaphorin 7A promotes angiogenesis in an experimental corneal neovascularization model. *Curr Eye Res* 2011;36(11):989-996.

14 Garcia-Areas R, Libreros S, Amat S, Keating P, Carrio R, Robinson P, Blieden C, Iragavarapu-Charyulu V. Semaphorin7A promotes tumor growth and exerts a pro-angiogenic effect in macrophages of mammary tumor-bearing mice. *Front Physiol* 2014;5:17.

15 Jiménez B, Volpert OV. Mechanistic insights on the inhibition of tumor angiogenesis. *J Mol Med* 2001;78(12):663-672.

16 Boudria A, Abou Faycal C, Jia T, Gout S, Keramidas M, Didier C, Lemaître N, Manet S, Coll JL, Toffart AC, Moro-Sibilot D, Albiges-Rizo C, Josserand V, Faurobert E, Brambilla C, Brambilla E, Gazzeri S, Eymin B. VEGF165b, a splice variant of VEGF-A, promotes lung tumor progression and escape from anti-angiogenic therapies through a β1 integrin/VEGFR autocrine loop. *Oncogene* 2019;38(7):1050-1066.

17 Cárdenas-Cantú E, Zavala J, Valenzuela J, Valdez-García JE. Molecular basis of pterygium development. *Seminars in Ophthalmology* 2014:1-17.

18 Black SA, Nelson AC, Gurule NJ, Futscher BW, Lyons TR. Semaphorin 7a exerts pleiotropic effects to promote breast tumor progression. *Oncogene* 2016;35(39):5170-5178.

19 Liu TJ, Guo JL, Wang HK, Xu X. Semaphorin-7A contributes to growth, migration and invasion of oral tongue squamous cell carcinoma through TGF-β-mediated EMT signaling pathway. *Eur Rev Med Pharmacol Sci* 2018;22(4):1035-1043.

20 Namavari A, Chaudhary S, Ozturk O, Chang JH, Yco L, Sonawane S, Katam N, Khanolkar V, Hallak J, Sarkar J, Jain S. Semaphorin 7a links nerve regeneration and inflammation in the cornea. *Invest Ophthalmol Vis Sci* 2012;53(8):4575-4585.

21 Khalfaoui T, Mkannez G, Colin D, Imen A, Zbiba W, Errais K, Anane R, Beltaief O, Zhioua R, Ben Hamida J, Lizard G, Ouertani-Meddeb A. Immunohistochemical analysis of vascular endothelial growth factor (VEGF) and p53 expression in pterygium from Tunisian patients. *Pathol Biol* 2011;59(3):137-141.

22 Lu CW, Hao JL, Yao L, Li HJ, Zhou DD. Efficacy of curcumin in inducing apoptosis and inhibiting the expression of VEGF in human pterygium fibroblasts. *Int J Mol Med* 2017;39(5):1149-1154.

23 Wang YC, Lin JY, Chen LX, Wang LM, Hao P, Han RF, Ying M, Li X. Expression of peroxiredoxin 2 and vascular endothelial growth factor receptor 2 in pterygium. *Cornea* 2017;36(7):841-844.

24 Feng QY, Hu ZX, Song XL, Pan HW. Aberrant expression of genes and proteins in pterygium and their implications in the pathogenesis. *Int J Ophthalmol* 2017;10(6):973-981.

25 Chen TT, Luque A, Lee S, Anderson SM, Segura T, Iruela-Arispe ML. Anchorage of VEGF to the extracellular matrix conveys differential signaling responses to endothelial cells. *J Cell Biol* 2010;188(4):595-609.

26 Jeong BY, Cho KH, Jeong KJ, Park YY, Kim JM, Rha SY, Park CG, Mills GB, Cheong JH, Lee HY. Rab25 augments cancer cell invasiveness through a β1 integrin/EGFR/VEGF-A/Snail signaling axis and expression of fascin. *Exp Mol Med* 2018;50(1):e435.

27 Scott GA, McClelland LA, Fricke AF. Semaphorin 7a promotes spreading and dendricity in human melanocytes through beta1-integrins. *J Invest Dermatol* 2008;128(1):151-161.

28 Bender RJ, Mac Gabhann F. Expression of VEGF and semaphorin genes define subgroups of triple negative breast cancer. *PLoS One* 2013;8(5):e61788.

29 Chang JH, Garg NK, Lunde E, Han KY, Jain S, Azar DT. Corneal neovascularization: an anti-VEGF therapy review. *Surv Ophthalmol* 2012;57(5):415-429.

30 Marcovich AL, Morad Y, Sandbank J, Huszar M, Rosner M, Pollack A, Herbert M, Bar-Dayan Y. Angiogenesis in pterygium: morphometric and immunohistochemical study. *Curr Eye Res* 2002;25(1):17-22.